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=>
=> dhis
DHIS IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> d his

     (FILE 'HOME' ENTERED AT 09:30:00 ON 10 MAR 2006)

     FILE 'DISSABS, 1MOBILITY, AGRICOLA, AQUASCI, BIOTECHNO, COMPENDEX,
     COMPUAB, CONF, CONFSCI, ELCOM, HEALSAFE, IMSDRUGCONF, LIFESCI, OCEAN,
     PAPERCHEM2, PASCAL, POLLUAB, SOLIDSTATE, ADISCTI, ADISINSIGHT, ADISNEWS,
     ANABSTR, ANTE, AQUALINE, BIOENG, BIOSIS, ...' ENTERED AT 09:30:19 ON 10
     MAR 2006
L1        850247 S (VLA1 OR (VLA (A) 1) OR ALPHA1 OR (ALPHA (A) 1) OR (VERY LATE
L2         28371 S L1 (S) (INFLAMMATION OR (RENAL (A) FIBROSIS) OR KIDNEY)
L3          5412 S L2 (S) (VIVO OR ADMINISTER? OR INJECT?)

     FILE 'DISSABS, AGRICOLA, AQUASCI, BIOTECHNO, COMPENDEX, HEALSAFE,
     LIFESCI, OCEAN, PASCAL, ADISCTI, ADISINSIGHT, ANTE, BIOENG, BIOSIS,
     BIOTECHDS, CABA, CAPLUS, CEABA-VTB, CROPU, DDFB, DGENE, DRUGB, DRUGU,
     EMBAL, EMBASE, ESBIOBASE, FEDRIP, GENBANK, IFIPAT, ...' ENTERED AT
     09:38:59 ON 10 MAR 2006
L4          4222 DUP REM L3 (1190 DUPLICATES REMOVED)
L5           290 S L4 (S) (INTEGRIN OR COLLAGEN)
L6            39 S L5 (S) (ALPHA (A) 1 (A) BETA (A) 1)

=>
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L6 ANSWER 21 OF 39 IFIPAT COPYRIGHT 2006 IFI on STN

AN 10202710 IFIPAT;IFIUDB;IFICDB

TITLE: METHOD FOR THE TREATMENT OF INFLAMMATORY DISORDERS; ADMINISTERING FUNCTION BLOCKING ANTIBODY OR A FRAGMENT ANTIBODY, CAPABLE OF BINDING AN EPITOPE OF VLA-1 TO PROVIDE A DECREASE IN ARTHRITIC SCORE OF ABOUT 65% OR GREATER WHEN COMPARED TO CONTROL ANTIBODY TREATED SUBJECT

INVENTOR(S): De Fougerolles; Antonin, Brookline, MA, US
Gotwals; Philip, West Roxbury, MA, US
Kotelianski; Victor, Boston, MA, US
Lobb; Roy, Westwood, MA, US

PATENT ASSIGNEE(S): Unassigned

PATENT ASSIGNEE PROBABLE: Biogen Idec MA Inc (Probable)

AGENT: John T. Li BIOGEN, INC., 14 Cambridge Center, Cambridge, MA, 02142, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2002146417	A1	20021010
APPLICATION INFORMATION:	US 2001-996738		20011130

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
CONTINUATION OF:	WO 2000-US15004	20000601	UNKNOWN

	NUMBER	DATE	
PRIORITY APPLN. INFO.:	US 1999-137038P	19990601	(Provisional)
	US 2000-185336P	20000229	(Provisional)
FAMILY INFORMATION:	US 2002146417	20021010	

DOCUMENT TYPE: Utility
Patent Application - First Publication

FILE SEGMENT: CHEMICAL
APPLICATION

NUMBER OF CLAIMS: 7 18 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1. **Collagen**-binding **integrins alpha** ***1*** **beta** 1 and alpha 2 beta 1 on activated leukocytes. (A). Flow cytometric analysis of **alpha** 1 and alpha 2 beta 1 **integrin** expression on IL-2activated splenocytes (d 11). Cells were labeled with either anti-**alpha** 1 mAb, anti-alpha 2 mAb, or non-binding control mAb (grey lines), and followed by FITC-anti-hamster immunoglobulin. (B) Effect of anti-**alpha** 1 and anti-alpha 2 mAbs on leukocyte adhesion to **collagen**. 105 IL-2 activated splenocytes were treated with indicated mAbs for 15 min before plating onto either type IV or type I **collagen**-coated wells for 1 h at 37 degrees C. Adhesion was calculated as illustrated in Example 1, and expressed as % adhesion relative to control mAbtreated cells. Adhesion assays were done in triplicate, and at least three independent experiments were performed. One representative experiment is shown.

FIG. 2. Effect of anti-**integrin** mAbs on the effector phase of delayed-type hypersensitivity. SRBC-sensitized mice were **injected** i.p. with the indicated mAbs 1 h prior to SRBC challenge. Footpad thickness was measured 20 h after antigen challenge, and results shown as % increase in footpad thickness +-SEM as illustrated in Example 2. These data represent a summary of eight experiments with n=79 (PBS), 68 (control hamster Ig), 68 (anti-**alpha** 1), 29 (anti-alpha 2), 18 (antialpha 1+anti-alpha 2), 45 (anti-alpha 4), 18 (anti-alpha 5), 20 (anti-alpha 6), and 10 (anti-beta 1). The mAbs used were: Ha4/8 (control hamster Ig group 2), Ha31/8 (anti-**alpha** 1), Hal/ 29 (anti-alpha 2), PS/2 (anti-alpha 4), 5H10-27 (anti-alpha 5), GoH3 (anti-alpha 6), and HM beta 1-1 (anti-beta 1).

FIG. 3. Effect of anti-**integrin** mAbs on the effector phase of contact hypersensitivity. FITC-sensitized mice were **injected** i. p. with the indicated mAbs 4 h prior to FITC challenge. Ear thickness was measured at

baseline and 24 h later, and results shown as % increase in ear thickness +-SEM as illustrated in Example 3. These data represent a summary of nine experiments with n=74 (PBS), 60 (control hamster Ig), 26 (anti-ICAM-1), 44 (anti-***alpha*** 1), 44 (anti-alpha 2), 38 (anti-**alpha** ***1*** +antialpha 2), 36 (anti-alpha 4), 16 (anti-alpha 5), 26 (antialpha 4+anti-alpha 5), 24 (anti-alpha 6), and 22 (anti-beta 1). The hamster mAbs used were: Ha4/8 (control hamster Ig group 2), Ha31/8 (anti-**alpha** ***1***), Hal/29 (anti-alpha 2), HM beta 1-1 (anti-beta 1), 3E2 (anti-ICAM-1); the rat mAbs used were: R3595 and R35-38 (control rat IgG2a and rat IgG2b, respectively), PS/2 (anti-alpha 4), 5H10-27 (anti-alpha 5), GoH3 (antialpha 6).

FIG. 4. Contact hypersensitivity responses in **alpha 1** -deficient mice compared to wild-type mice. FITC-sensitized mice were ***injected*** i.p. with indicated mAbs 4 h prior to FITC challenge. Ear thickness was measured at baseline and 24 h later, and results shown as % increase in ear thickness +-SEM as illustrated in Example 4. Groups of four to five mice per condition were used, and all experiments were performed a minimum of three times. One representative experiment is shown.

FIG. 5. Effect of anti-**alpha 1** and anti-alpha 2 mAbs on croton oil-induced non-specific **inflammation**. Mice were ***injected*** i.p. with indicated mAbs 4 h prior to ear painting with croton oil. Ear thickness was measured at baseline and 24 h later, and results shown as % increase in ear thickness +-SEM as illustrated in Example 5. Groups of four to five mice per condition were used, and all experiments were performed a minimum of three times. One representative experiment is shown.

FIG. 6. Effect of anti-**alpha 1** and alpha 2 mAbs in ***collagen*** mAbinduced arthritis. Mice were **injected** i.p. with anti-**collagen** mAbs at d 0, followed by LPS on day 3. Mice were ***injected*** i.p. with indicated mAbs every 3rd day starting on d 0. Clinical arthritis was apparent 2-3 d following LPS **injection** and continued for several weeks. Each limb was evaluated on a 0 to 4 scale every 3rd day as illustrated in Example 6 and results are expressed as the mean arthritic score between d 9 and d 15 (+-SEM) of all four limbs. These data represent a summary of four experiments with each experiment consisting of groups of three to four mice per condition.

FIG. 7. Administration of anti-**alpha 1** or anti-alpha 2 mAbs inhibits leukocyte infiltration into footpads during a DTH response. The experiment was performed as described in FIG. 2. Footpads were excised 20 h following antigen challenge and tissue sections stained with hematoxylin and eosin. Tissue sections are from footpads of either unchallenged mice (A) or SRBC-sensitized mice challenged with SRBC (B-H). Mice were treated 1 h prior to challenge with either PBS (B), control hamster Ig (C, G), anti-**alpha** ***1*** (D), anti-alpha 2 (E) or a combination of anti-**alpha** ***1*** and anti-alpha 2 mAbs (F,H). Magnification: x 100 (A-F), x 400 (G-H).

FIG. 8. **alpha 1 beta 1** is expressed on infiltrating leukocytes in footpads during a DTH response. Immunohistochemical staining of infiltrating leukocytes from an untreated inflamed footpad 20 h after antigen challenge. (A). Serial sections stained directly with Alexa488-conjugated control mAb and anti-**alpha 1** mAb. (B). Dual immunofluorescent staining with Alexa488conjugated anti-**alpha** ***1*** mAb and Phycoerythrin (PE)-conjugated cell lineage-specific mAbs. PE-conjugated mAbs utilized were specific for granulocytes/monocytes (anti-CD11b), neutrophils (anti-Ly6G/Gr-1), and T lymphocytes (anti-CD3). Magnification: x 400.

FIG. 9. Effect of anti-**alpha 1** and alpha 2 mAbs in ***collagen*** mAbinduced arthritis. (A). Preventative treatment of mice with either anti-**alpha 1** or anti-alpha 2 mAb decreases arthritic score. Mice were treated with anti-**collagen** mAbs at d 0, followed by LPS on d 3. Arthritis was apparent by d 6 and continued for several weeks. Mice were treated with the indicated mAbs every 3rd day starting on d 0. Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score between d 9 and d 15 (+-SEM) of all four limbs (maximum score of 16). Groups of 4 mice per condition were used; the average of 12 experiments is shown. (B). **alpha 1**-deficient mice have a reduced arthritic score comparable to anti-**alpha 1** mAb-treated wild-type mice. Experimental details and scoring are as outlined above. Groups of 4 mice per condition were used; the average of 2 experiments is shown.

FIG. 10. Effect of anti-**alpha 1** mAb treatment on the

immunopathology of arthritic joints. Anti-alpha 1 mAb treatment reduces leukocytic infiltration, adherence of cells to joint surfaces, and cartilage destruction as evidenced by proteoglycan loss. Hind limbs from normal mice (A-D) or arthritic mice (d 8) receiving either control hamster Ig (E-H) or anti-alpha 1 mAb treatment. (I-L).
Limbs were photographed (A, E, I), excised, and tissue sections stained either with hematoxylin/eosin (B-C, F-G, J-K) or with toluidine blue to detect proteoglycan (D, H, L). Magnification: x 16 (B, F, J); x 160 (C, G, K); x 200 (D, H, L).
FIG. 11. Development of arthritis is delayed in the absence of lymphocytes and inhibition of arthritis by anti-alpha 1 mAb occurs in the absence of lymphocytes. Wild-type B6,129 or RAG-1deficient B6,129 mice were treated with anti-collagen mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated with the indicated mAbs every 3rd day starting on day 0. Each rd limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.
FIG. 12. Dose response of anti-alpha 1 mAb inhibition of arthritis. Wild-type Balb/c mice were treated with anticollagen mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated i.p. with the indicated dose of either Ha4/8 (isotype control) or Ha31/8 (anti-alpha 1 mAbs every 3rd day starting on day 0. Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.
FIG. 13. Therapeutic treatment with anti-alpha 1 mAb can decrease arthritic score. Wild-type Balb/c mice were treated with anti-***collagen*** mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated i.p. with mAbs (250 ug) or Ig fusion protein (200 ug) every 3d day starting on day 4. Mice received either mAb (Ha4/8 isotype control or Ha31/8 anti-alpha ***1***), Ig fusion protein (Isotype control Ig or TNF-R55-Ig) or a combination of both (250 ug Ha31/8 and 200 ug TNF-R55-Ig). Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.
FIG. 14. Location of the Epitope for the anti-alpha 1 domain Blocking mAbs. A. Amino acid sequence of the rat (top) and human (below) ***alpha*** 1-I domain. The residues that comprise the MIDAS (metal ion dependent adhesion site) motif are shown in bold. The human amino acids that replaced the corresponding rat residues (RAH) are shown below the rat sequence in the boxed region. For clarity, residue numbering in the text refers to this figure. B. Increasing concentrations of mAb AJH10 were bound to plates coated with 30 mu g/ml human (circles), rat (triangles) or R Delta H (squares) ***alpha*** 1-I domain. Data shown is representative of three experiments.
FIG. 15. Amino acid sequence of the human alpha 1-I domain.

DESCRIPTION OF FIGURES:

FIG. 16. Identification of a blocking mAb to the alpha 1-I domain. A. Increasing concentration of mAbs AEF3 (triangles) or AJH10 (circles) were bound to plates coated with 30 mu g/ml alpha 1-I domain. B. The alpha 1-I domain was treated with increasing concentrations of mAb AJH10 (diamonds) or mAb BGC5 (squares) and bound collagen IV (2 mu g/ml) coated plates. C. K562-alpha 1 cell were treated with increasing concentration of mAbs AEF3(triangles) or AJH10 (circles) and bound to collagen IV (5 mu g/ml) coated plates. 45-50% of cells added to each well adhered to collagen IV. Data shown is representative of three independent experiments.
FIG. 17. Species Cross-reactivity of the blocking mAbs. A. Detergents lysates from (1) sheep vascular smooth muscle, (2) human leukemia K562-alpha 1 cells or (3) purified R Delta H GST-I domain; (4) Rat GST-alpha 1 I domain; and (5) human GSTalpha 1 I domain were separated by 10-20% SDS-PAGE under nonreducing conditions, and immunoblotted with function-blocking mAb AJH10. Molecular weight markers are shown on the left; nonreduced alpha 1 beta 1 integrin migrates at 180 kDa; GST-I domain migrates at 45 kDa. B. Rabbit vascular smooth muscle cells were incubated with either mAb AJH10 (bottom) or murine IgG control (top) and analyzed by fluorescence activated cell sorter (FACS).
FIG. 18. The alpha 1-I domain binds collagen. A. Increasing concentrations of the human alpha 1-I domain were bound to plates previously coated with 1 mu

g/ml collagen I (squares) or collagen IV (circles). Values shown have been corrected for background binding to BSA. B. 2 mu g/ml human alpha 1-I domain was mixed with increasing concentration of an anti-human alpha 1 integrin antibody 5E8D9 (squares) or an anti-human alpha 2integrin antibody A2IIE10 (circles), and then bound to plates previously coated with 1 mu g/ml collagen IV. C. Plates were coated with 1 mu g/ml collagen IV or 3% BSA. alpha 1-I domain (2 mu g/ml) was subsequenctly bound to coated plates plates in the presence of 1 mM Mn2+, 1 mM Mg2+, or 5 mM EDTA. Data shown is representative of three independent experiments. !

AB A method for the treatment of inflammatory disorders is disclosed, particularly the treatment of arthritis. The method comprises the administration of a function blocking antibody which is capable of binding an epitope of VLA-1.

GI 18 Figure(s).

FIG. 1. **Collagen**-binding **integrins alpha** 1 **beta** 1 and alpha 2 beta 1 on activated leukocytes. (A). Flow cytometric analysis of **alpha** 1 and alpha 2 beta 1 **integrin** expression on IL-2activated splenocytes (d 11). Cells were labeled with either anti-**alpha** 1 mAb, anti-alpha 2 mAb, or non-binding control mAb (grey lines), and followed by FITC-anti-hamster immunoglobulin. (B) Effect of anti-**alpha** 1 and anti-alpha 2 mAbs on leukocyte adhesion to **collagen**. 105 IL-2 activated splenocytes were treated with indicated mAbs for 15 min before plating onto either type IV or type I **collagen**-coated wells for 1 h at 37 degrees C. Adhesion was calculated as illustrated in Example 1, and expressed as %. . . . done in triplicate, and at least three independent experiments were performed. One representative experiment is shown.

FIG. 2. Effect of anti-**integrin** mAbs on the effector phase of delayed-type hypersensitivity. SRBC-sensitized mice were **injected** i.p. with the indicated mAbs 1 h prior to SRBC challenge. Footpad thickness was measured 20 h after antigen challenge,. . . illustrated in Example 2. These data represent a summary of eight experiments with n=79 (PBS), 68 (control hamster Ig), 68 (anti-**alpha** 1), 29 (anti-alpha 2), 18 (antialpha 1+anti-alpha 2), 45 (anti-alpha 4), 18 (anti-alpha 5), 20 (anti-alpha 6), and 10 (anti-beta 1). The mAbs used were: Ha4/8 (control hamster Ig group 2), Ha31/8 (anti-**alpha** 1), Hal/ 29 (anti-alpha 2), PS/2 (anti-alpha 4), 5H10-27 (anti-alpha 5), GoH3 (anti-alpha 6), and HM beta 1-1 (anti-beta 1).

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injection and continued for several weeks. Each limb was evaluated on a 0 to 4 scale every 3rd day as illustrated. . . of four experiments with each experiment consisting of groups of three to four mice per condition.

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FIG. 9. Effect of anti-alpha 1 and alpha 2 mAbs in collagen mAbinduced arthritis. (A). Preventative treatment of mice with either anti-alpha 1 or anti-alpha 2 mAb decreases arthritic score. Mice were treated with anti-collagen mAbs at d 0, followed by LPS on d 3. Arthritis was apparent by d 6 and continued for several. . . (maximum score of 16). Groups of 4 mice per condition were used; the average of 12 experiments is shown. (B). alpha 1-deficient mice have a reduced arthritic score comparable to anti-alpha 1 mAb-treated wild-type mice. Experimental details and scoring are as outlined above. Groups of 4 mice per condition were used; the average of 2 experiments is shown.

FIG. 10. Effect of anti-alpha 1 mAb treatment on the immunopathology of arthritic joints. Anti-alpha 1 mAb treatment reduces leukocytic infiltration, adherence of cells to joint surfaces, and cartilage destruction as evidenced by proteoglycan loss. Hind limbs from normal mice (A-D) or arthritic mice (d 8) receiving either control hamster Ig (E-H) or anti-alpha 1 mAb treatment. (I-L). Limbs were photographed (A, E, I), excised, and tissue sections stained either with hematoxylin/eosin (B-C, F-G, J-K). . . (D, H, L).

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FIG. 13. Therapeutic treatment with anti-alpha 1 mAb can decrease arthritic score. Wild-type Balb/c mice were treated with anti-collagen mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several. . . fusion protein (200 ug) every 3d day starting on day 4. Mice received either mAb (Ha4/8 isotype control or Ha31/8 anti-alpha 1), Ig fusion protein (Isotype control Ig or TNF-R55-Ig) or a combination of both (250 ug Ha31/8 and 200 ug TNF-R55-Ig).. . . (maximum score of 4). Groups of 4 mice per condition were used.

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The human amino. . . mAb AJH10 were bound to plates coated with 30 mu g/ml human (circles), rat (triangles) or R Delta H (squares) alpha 1-I domain. Data shown is representative of three experiments.

FIG. 15. Amino acid sequence of the human alpha 1-I domain.

L6 ANSWER 28 OF 39 USPATFULL on STN

ACCESSION NUMBER:	2005:260856 USPATFULL
TITLE:	Method for the treatment of inflammatory disorders
INVENTOR(S):	Gotwals, Philip, Needham, MA, UNITED STATES DeFougerolles, Antonin, Brookline, MA, UNITED STATES Lobb, Roy, Westwood, MA, UNITED STATES Kotelianski, Victor, Boston, MA, UNITED STATES
PATENT ASSIGNEE(S):	BIOGEN IDEC, INC., Cambridge, MA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005226877	A1	20051013
APPLICATION INFO.:	US 2005-108581	A1	20050418 (11)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2001-996738, filed on 30 Nov 2001, PENDING Continuation of Ser. No. WO 2000-US15004, filed on 1 Jun 2000, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-185336P	20000229 (60)
	US 1999-137038P	19990601 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	Kevin J. McGough, Esquire, Coleman, Sudol & Sapone, P.C., 714 Colorado Avenue, Bridgeport, CT, 06605-1601, US
NUMBER OF CLAIMS:	20
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	19 Drawing Page(s)
LINE COUNT:	1588

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for the treatment of inflammatory disorders is disclosed, particularly the treatment of arthritis. The method comprises the administration of a function blocking antibody which is capable of binding an epitope of VLA-1.

DETD Expression andfunctional blockade of .**alpha**.1. **beta**.1 and α2β1 on activated leukocytes. Given the key role leukocytes play in **inflammation**, we decided to test whether anti-.**alpha**.1 and anti-α2 mAbs were capable of blocking leukocyte adhesion to **collagens**. In order to obtain leukocytes expressing high levels of both .**alpha** .1 and α2, murine T cells were stimulated in vitro with IL-2 for 7-12 d. These cells expressed high levels of both . **alpha**.1 and α2 (FIG. 1A), and bound well to both **collagen** type IV and type I-coated surfaces (FIG. 1B). Adhesion to type IV **collagen** was partially inhibited by anti-. **alpha**.1 mAb alone and was not inhibited by anti-α2 mAb alone. In contrast, adhesion to type I **collagen** was completely inhibited by anti-α2 mAb and anti-.**alpha**.1 mAb alone showed only partial inhibition. Both anti-β1 mAb and the combination of anti-. **alpha**.1 and anti-α2 mAbs completely inhibited adhesion to types I and IV **collagen**. Having demonstrated that the .**alpha**.1.**beta**.1 and α2β1 **integrins** are expressed on activated T cells and that anti-.**alpha**.1 and α2 mAbs are able to functionally block leukocyte adhesion to **collagens**, we used these mAbs to investigate the in **vivo** role of these **integrins** in animal models of inflammatory disorders.

L6 ANSWER 18 OF 39 IFIPAT COPYRIGHT 2006 IFI on STN

AN 10574429 IFIPAT;IFIUDB;IFICDB

TITLE: ANTIBODIES TO VLA-1; CRYSTAL STRUCTURE OF THE COMPLEX BETWEEN ONE SUCH ANTIBODY AND THE ALPHA 1-I DOMAIN OF VLA-1, AND TO THE USE OF THIS STRUCTURAL INFORMATION FOR COMPUTATIONAL DRUG DESIGN

INVENTOR(S): Garber; Ellen A, Cambridge, MA, US
Karpusas; Michael, Upper Darby, PA, US
Lyne; Paul D, Arlington, MA, US
Saldanha; Jose William B, Middlesex, GB

PATENT ASSIGNEE(S): Unassigned
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AGENT: FISH & NEAVE, 1251 AVENUE OF THE AMERICAS, 50TH FLOOR, NEW YORK, NY, 10020-1105, US

	NUMBER	PK	DATE	
PATENT INFORMATION:	US 2004081651	A1	20040429	
APPLICATION INFORMATION:	US 2003-474832		20031014	
	WO 2002-US11521		20020412	
			20031014	PCT 371 date
			20031014	PCT 102(e) date
FAMILY INFORMATION:	US 2004081651		20040429	

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION

NUMBER OF CLAIMS: 42 23 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1. **Collagen**-binding **integrins alpha** ***1*** **beta 1** and alpha 2 beta 1 on activated leukocytes. (A). Flow cytometric analysis of **alpha 1** and alpha 2 beta 1 **integrin** expression on IL-2activated splenocytes (d 11). Cells were labeled with either anti-**alpha 1** mAb, anti-alpha 2 mAb, or non-binding control mAb (grey lines), and followed by FITC-anti-hamster immunoglobulin. (B) Effect of anti-al and anti-a2 mAbs on leukocyte adhesion to **collagen**. 105 IL-2 activated splenocytes were treated with indicated mAbs for 15 min before plating onto either type IV or type I **collagen**-coated wells for 1 h at 37 degrees C. Adhesion was calculated as illustrated in Example 1, and expressed as % adhesion relative to control mab-treated cells. Adhesion assays were done in triplicate, and at least three independent experiments were performed. One representative experiment is shown.

FIG. 2. Effect of anti-**integrin** mAbs on the effector phase of delayed-type hypersensitivity. SRBC-sensitized mice were **injected** i.p. with the indicated mAbs 1 h prior to SRBC challenge. Footpad thickness was measured 20 h after antigen challenge, and results shown as % increase in footpad thickness +-SEM as illustrated in Example 2. These data represent a summary of eight experiments with n=79 (PBS), 68 (control hamster Ig), 68 (anti-**alpha 1**), 29 (anti-alpha 2), 18 (antialpha 1+anti-alpha 2), 45 (anti-alpha 4), 18 (anti-alpha 5), 20 (anti-alpha 6), and 10 (anti-beta 1). The mAbs used were: Ha4/8 (control hamster Ig group 2), Ha31/8 (anti-**alpha 1**), Ha1/ 29 (anti-alpha 2), PS/2 (anti-alpha 4), 5H10-27 (anti-alpha 5), GoH3 (anti-alpha 6), and HM beta 1-1 (anti-beta 1).

FIG. 3. Effect of anti-**integrin** mAbs on the effector phase of contact hypersensitivity. FITC-sensitized mice were **injected** i. p. with the indicated mAbs 4 h prior to FITC challenge. Ear thickness was measured at baseline and 24 h later, and results shown as % increase in ear thickness +-SEM as illustrated in Example 3. These data represent a summary of nine experiments with n=74 (PBS), 60 (control hamster Ig), 26 (anti-ICAM-1), 44 (anti-***alpha*** 1), 44 (anti-alpha 2), 38 (anti-**alpha** ***1*** +antialpha 2), 36 (anti-alpha 4), 16 (anti-alpha 5), 26 (antialpha 4+anti-alpha 5), 24 (anti-alpha 6), and 22 (anti-beta 1). The hamster mAbs used were: Ha4/8 (control hamster Ig group 2), Ha31/8 (anti-**alpha** ***1***), Ha1/29 (anti-alpha 2), HM beta 1-1 (anti-beta 1), 3E2 (anti-ICAM-1); the rat mAbs used were: R3595 and R35-38 (control rat IgG2a and

rat IgG2b, respectively), PS/2 (anti-alpha 4), 5H10-27 (anti-alpha 5), GoH3 (anti-alpha 6).

FIG. 4. Contact hypersensitivity responses in alpha 1 -deficient mice compared to wild-type mice. FITC-sensitized mice were ***injected*** i.p. with indicated mAbs 4 h prior to FITC challenge. Ear thickness was measured at baseline and 24 h later, and results shown as % increase in ear thickness +-SEM as illustrated in Example 4. Groups of four to five mice per condition were used, and all experiments were performed a minimum of three times. One representative experiment is shown.

FIG. 5. Effect of anti-alpha 1 and anti-alpha 2 mAbs on croton oil-induced non-specific inflammation. Mice were ***injected*** i.p. with indicated mAbs 4 h prior to ear painting with croton oil. Ear thickness was measured at baseline and 24 h later, and results shown as % increase in ear thickness +-SEM as illustrated in Example 5. Groups of four to five mice per condition were used, and all experiments were performed a minimum of three times. One representative experiment is shown.

FIG. 6. Effect of anti-alpha 1 and alpha 2 mAbs in ***collagen*** mAbinduced arthritis. Mice were injected i.p. with anti-collagen mAbs at d 0, followed by LPS on day 3. Mice were ***injected*** i.p. with indicated mAbs-every 3rd day starting on d 0. Clinical arthritis was apparent 2-3 d following LPS injection and continued for several weeks. Each limb was evaluated on a 0 to 4 scale every 3rd day as illustrated in Example 6 and results are expressed as the mean arthritic score between d 9 and d 15 (+-SEM) of all four limbs. These data represent a summary of four experiments with each experiment consisting of groups of three to four mice per condition.

FIG. 7. Effect of anti-alpha 1 and alpha 2 mAbs in ***collagen*** mAbinduced arthritis. A. Preventative treatment of mice with either anti-alpha 1 or anti-a2 mAb decreases arthritic score. Mice were treated with anti-collagen mAbs at d 0, followed by LPS on d 3. Arthritis was apparent by d 6 and continued for several weeks. Mice were treated with the indicated mAbs every 3rd day starting on d 0. Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score between d 9 and d 15 (+-SEM) of all four limbs (maximum score of 16). Groups of 4 mice per condition were used; the average of 12 experiments is shown. B. alpha 1deficient mice have a reduced arthritic score comparable to anti-alpha 1 mAb-treated wild-type mice. Experimental details and scoring are as outlined above. Groups of 4 mice per condition were used; the average of 2 experiments is shown.

FIG. 8. Development of arthritis is delayed in the absence of lymphocytes and inhibition of arthritis by anti-alpha 1 mAb occurs in the absence of lymphocytes. Wild-type B6,129 or RAG-1deficient B6,129 mice were treated with anti-collagen mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated with the indicated mAbs every 3rd day starting on day 0. Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.

FIG. 9. Dose response of anti-al mAb inhibition of arthritis. Wild-type Balb/c mice were treated with anti-collagen mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated i.p. with the indicated dose of either Ha4/8 (isotype control) or Ha31/8 (anti-alpha 1) mAbs every 3rd day starting on day 0. Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.

FIG. 10. Therapeutic treatment with anti-alpha 1 mAb can decrease arthritic score. Wild-type Balb/c mice were treated with anti-***collagen*** mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated i.p. with mAbs (250 mu g) or Ig fusion protein (200 mu g) every 3rd day starting on day 4. Mice received either mAb (Ha4/8 isotype control or Ha31/8 antialpha 1), Ig fusion protein (Isotype control Ig or TNF-R55-Ig) or a combination of both (250 ug Ha31/8 and 200 ug TNF-R55-Ig). Each limb was evaluated and scored on a 0 to 4 scale every 3rd day. Results are expressed as the mean arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.

FIG. 11. Location of the Epitope for the anti-alpha 1 I domain Blocking nabs. A. Amino acid sequence of the rat (top; SEQ ID NO:63) and human (below; SEQ ID NO:64) alpha 1-I domain. The residues

that comprise the MIDAS (metal ion dependent adhesion site) motif are shown in bold. The human amino acids that replaced the corresponding rat residues (R Delta H) are shown below the rat sequence in the boxed region. For clarity, residue numbering in the text refers to this figure,, unless otherwise designated, e.g., as crystal numbering. B. Increasing concentrations of mAb AJH10 (ATCC No. PTA-3580; deposited under the Budapest Treaty with the American Type Culture Collection, Manassas, Va., USA on Aug. 2, 2001) were bound to plates coated with 30 mu g/ml human (circles), rat (triangles) or R Delta H (squares) **alpha 1**-I domain. Data shown is representative of three experiments.

FIG. 12. Amino acid sequence of the human **alpha 1**-I domain (SEQ ID NO:64).

FIG. 13. Identification of a blocking mab to the **alpha 1**-I domain. A. Increasing concentration of mAbs AEF3 (triangles) or AJH10 (circles) were bound to plates coated with 30 mu g/ml **alpha 1**-I domain. B. The **alpha 1**-I domain was treated with increasing concentrations of mAb AJH10 (diamonds) or mAb BGC5 (squares) and bound ***collagen*** IV (2 mu g/ml) coated plates. C. K562-**alpha** ***1*** cell were treated with increasing concentration of mAbs AEF3(triangles) or AJH10 (circles) and bound to **collagen** IV (5 mu g/ml) coated plates. 45-50% of cells added to each well adhered to ***collagen*** IV. Data shown is representative of three independent experiments.

FIG. 14. Species Cross-reactivity of the blocking mAbs analyzed by fluorescence activated cell sorter (FACS). Rabbit vascular smooth muscle cells were incubated with either mAb AJH10 (bottom) or murine IgG control (top) and analyzed by fluorescence activated cell sorter (FACS).

FIG. 15. The **alpha 1**-I domain binds **collagen**. A. Increasing concentrations of the human **alpha 1**-I domain were bound to plates previously coated with 1 mu g/ml **collagen** I (squares) or **collagen** IV (circles). Values shown have been corrected for background binding to BSA. B. 2 mu g/ml human **alpha 1**-I domain was mixed with increasing concentration of an anti-human **alpha** ***1*** **integrin** antibody 5E8D9 (squares) or an anti-human alpha 2integrin antibody A2IIE10 (circles), and then bound to plates previously coated with 1 mu g/ml **collagen** IV. C. Plates were coated with 1 mu g/ml **collagen** IV or 3% BSA. **alpha 1**-I domain (2 mu g/ml) was subsequenctly bound to coated plates plates in the presence of 1 mM Mn2+, 1 mM Mg2+, or 5 mM EDTA. Data shown is representative of three independent experiments.

FIG. 16. Characterization of Humanized AQC2 Forms. mAQC2 (triangles), chAQC2 (circles), hAQC2 (inverted triangles) and hAQC2' (squares) were evaluated.

FIG. 17. Characterization of Humanized AQC2 Forms by FACS.

FIG. 18. Characterization of Humanized AQC2 Forms by FACS.

DESCRIPTION OF FIGURES:

FIG. 19. Atomic structure coordinates for the alpha 1-I domain/ Fab complex, as derived by X-ray crystallography from crystals of that complex in Protein Data Bank (PDB) format. The coordinates of the two complexes in the asymmetric unit are listed as follows.

FIG. 20. I domain-Fab complex. A. Ribbon diagram of the I domainFab complex. The I domain is colored green and the antibody heavy and light chain yellow and blue, respectively. The Mn+2 ion is the white colored sphere. B. Close-up of the MIDAS (Metal-Ion-Dependent-Adhesion-Site) site showing the coordination of the metal ion (white sphere) by Asp101 (crystal numbering). The protein backbones are shown as ribbons and the side chains in the ball-and-stick representation. The blue cylinders represent interactions between the metal ion and protein atoms. The thin lines represent H-bonds. FIG. 20 was made with the software program RIBBONS (Carson, 1991, J. Appl. Cryst. 24:958-961).

FIG. 21. A diagram of a system used to carry out the instructions encoded by the storage medium of FIGS. 22 and 23.

FIG. 22. A cross section of a magnetic storage medium.

FIG. 23. A cross section of an optically-readable data storage medium.!

AB Antibodies that specifically bind to VLA-1 integrin and methods of using these antibodies to treat immunological disorders in a subject. Also included are crystal structures of complexes formed by VLA-1 antibodies and their ligands, and VLA-1 antagonists and agonists identified by using the structure coordinates of these structures.

GI 23 Figure(s).

FIG. 1. **Collagen**-binding **integrins alpha**

1 beta 1 and alpha 2 beta 1 on activated leukocytes. (A). Flow cytometric analysis of alpha 1 and alpha 2 beta 1 integrin expression on IL-2activated splenocytes (d 11). Cells were labeled with either anti-alpha 1 mAb, anti-alpha 2 mAb, or non-binding control mAb (grey lines), and followed by FITC-anti-hamster immunoglobulin. (B) Effect of anti-al and anti-a2 mAbs on leukocyte adhesion to collagen. 105 IL-2 activated splenocytes were treated with indicated mAbs for 15 min before plating onto either type IV or type I collagen-coated wells for 1 h at 37 degrees C. Adhesion was calculated as illustrated in Example 1, and expressed as %. . . were done in triplicate, and at least three independent experiments were performed. One representative experiment is shown.

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FIG. 8. Development of arthritis is delayed in the absence of lymphocytes

and inhibition of arthritis by anti-**alpha** 1 mAb occurs in the absence of lymphocytes. Wild-type B6,129 or RAG-1deficient B6,129 mice were treated with anti-**collagen** mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several. . . mice per condition were used.

FIG. 9. Dose response of anti-al mAb inhibition of arthritis. Wild-type Balb/c mice were treated with anti-**collagen** mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several weeks. Mice were treated i.p. with the indicated dose of either Ha4/8 (isotype control) or Ha31/8 (anti-**alpha** 1) mAbs every 3rd day starting on day 0. Each limb was evaluated and scored on a 0 to 4 scale. . . arthritic score per limb (maximum score of 4). Groups of 4 mice per condition were used.

FIG. 10. Therapeutic treatment with anti-**alpha** 1 mAb can decrease arthritic score. Wild-type Balb/c mice were treated with anti-**collagen** mAbs at day 0, followed by LPS on day 3. Arthritis was apparent by day 6 and continued for several. . . limb (maximum score of 4). Groups of 4 mice per condition were used.

FIG. 11. Location of the Epitope for the anti-**alpha** 1 I domain Blocking nabs. A. Amino acid sequence of the rat (top; SEQ ID NO:63) and human (below; SEQ ID NO:64) **alpha** 1-I domain. The residues that comprise the MIDAS (metal ion dependent adhesion site) motif are shown in bold. The human amino. . . 2, 2001) were bound to plates coated with 30 mu g/ml human (circles), rat (triangles) or R Delta H (squares) **alpha** 1-I domain. Data shown is representative of three experiments.

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FIG. 14. Species Cross-reactivity of the blocking mAbs analyzed by fluorescence activated. . . with either mAb AJH10 (bottom) or murine IgG control (top) and analyzed by fluorescence activated cell sorter (FACS).

FIG. 15. The **alpha** 1-I domain binds **collagen** . A. Increasing concentrations of the human **alpha** 1-I domain were bound to plates previously coated with 1 mu g/ml **collagen** I (squares) or **collagen** IV (circles). Values shown have been corrected for background binding to BSA. B. 2 mu g/ml human **alpha** 1-I domain was mixed with increasing concentration of an anti-human **alpha** 1 **integrin** antibody 5E8D9 (squares) or an anti-human alpha 2integrin antibody A2IIE10 (circles), and then bound to plates previously coated with 1 mu g/ml **collagen** IV. C. Plates were coated with 1 mu g/ml **collagen** IV or 3% BSA. **alpha** 1-I domain (2 mu g/ml) was subsequenctly bound to coated plates plates in the presence of 1 mM Mn2+, 1 mM. . .

L6 ANSWER 19 OF 39 IFIPAT COPYRIGHT 2006 IFI on STN

AN 10570379 IFIPAT;IFIUDB;IFICDB

TITLE: METHODS AND COMPOSITIONS RELATING TO ISOLEUCINE BOROPROLINE COMPOUNDS; ABNORMAL CELL PROLIFERATION; INFECTIOUS DISEASES; INJECTION, ENTERICALLY COATED ORAL ADMINISTRATION

INVENTOR(S): Adams; Sharlene, Waltham, MA, US
Jesson; Michael I., Hopedale, MA, US
Jones; Barry, Cambridge, MA, US
Miller; Glenn T., Merrimac, MA, US

PATENT ASSIGNEE(S): Point Therapeutics, Inc., Boston, MA, US

AGENT: Maria A. Trevisan Wolf, Greenfield & Sacks, P.C., 600

Atlantic Avenue, Boston, MA, 02210, US

	NUMBER	PK	DATE	
PATENT INFORMATION:	US 2004077601	A1	20040422	
APPLICATION INFORMATION:	US 2003-616694		20030709	
	NUMBER		DATE	
PRIORITY APPLN. INFO.:	US 2002-394856P		20020709	(Provisional)
	US 2002-414978P		20021001	(Provisional)
	US 2003-466435P		20030428	(Provisional)
FAMILY INFORMATION:	US 2004077601		20040422	

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION

PARENT CASE DATA:

This application claims priority under 35 U.S.C. section 119(e) to U.S. Provisional Application Serial No. 60/394,856, entitled "BOROPROLINE COMPOUND COMBINATION THERAPY", filed on Jul. 9, 2002; U.S. Provisional Application Serial No. 60/414,978, entitled "BOROPROLINE COMPOUND COMBINATION THERAPY", filed on Oct. 1, 2002; and U.S. Provisional Application Serial No. 60/ 466,435, entitled "BOROPROLINE COMPOUND COMBINATION THERAPY", filed on Apr. 28, 2003, each of which is herein incorporated by reference in its entirety.

NUMBER OF CLAIMS: 484 2 Figure(s).
DESCRIPTION OF FIGURES:
FIG. 1. Comparison of the ability of Ile-boroPro to stimulate chemokine production in vivo when administered orally or by subcutaneous injection. Ile-boroPro was administered to mice by oral gavages (open symbols) or by subcutaneous injection (closed symbols) at the doses indicated on the abscissa. Two hours after administration, serum samples were obtained and assayed for DPP-IV activity (Delta / up-triangle-filled), using the fluorogenic substrate Ala-Pro-7-amino-4trifluoromethyl coumarin, and the chemokine KC (large-circle / circle-solid) by ELISA.
FIG. 2. Comparison of the anti-tumor activity of Ile-boroPro administered orally or by subcutaneous injection in the WEHI 164 mouse tumor model. Mice were inoculated subcutaneously with 4 x 106 tumor cells and administered Ile-boroPro twice daily from day 2 to day 19 after tumor inoculation, either by oral gavage (open bars) or subcutaneous injection (solid bars). Control mice received saline (hatched bar). The data represent mean tumor volumes+-SEM (n=10) were recorded on day 20. Treatment with all 3 doses of Ile-boroPro resulted in significant reduction in tumor sizes (oral administration: P less-than 0.05; subcutaneous administration: P less-than 0. 00005).

AB A method for treating subjects with, inter alia, abnormal cell proliferation or infectious disease. Compositions containing Ile-boroPro compounds are also provided. The invention embraces the use of these compounds alone or in combination with other therapeutic agents.

ACLM 256. The method of claim 192, 194, 198 **or** 203, **wherein** the **cancer antigen is a cancer testis (CT) antigen.**

L6 ANSWER 13 OF 39 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2000-04343 BIOTECHDS
TITLE: Novel methods for treating kidney disorders using an alpha-1-beta-1 integrin receptor-inhibitor and TGF-beta-1-inhibitor;
integrin receptor-inhibitor and transforming growth factor-inhibitor for use in therapy
AUTHOR: Cosgrove D
PATENT ASSIGNEE: Boys-Town-Nat.Res.Hosp.Omaha
LOCATION: Omaha, NE, USA.
PATENT INFO: WO 9961040 2 Dec 1999
APPLICATION INFO: WO 1999-US11073 19 May 1999
PRIORITY INFO: US 1998-150485 9 Sep 1998; US 1998-86587 22 May 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2000-116327 [10]
AB Using **alpha-1,beta-1 integrin** receptor-inhibitor (I) and transforming growth factor-beta-1-inhibitor (II) for therapy of **kidney** disorders is claimed. (I) is given to a patient to limit a **kidney** disorder. Also claimed are: delaying onset of and/or slowing progression of Alport Syndrome (AS) in a patient by **administering** an agent that inhibits signal transduction via an **alpha-1, beta-1 integrin** receptor (A1B1IR) of a **kidney** cell or by blocking an A1B1IR binding site on the surface of a patient's **kidney** cell; delaying onset of and/or slowing progression of **kidney** disease in insulin-dependent diabetes mellitus (IDDM) in a patient by **administering** an agent that inhibits signal transduction via an A1B1IR of a **kidney** cell; delaying onset of and/or slowing progression of a **kidney** disease in IDDM in a patient by blocking an A1B1IR binding site on the surface of a patient's **kidney** cell; limiting **kidney** fibrosis in a patient; a mouse model for **kidney** disease, where the mouse does not express normal **collagen** type 4 in glomerular basement membrane and does not express A1B1IR; drug screening; and limiting matrix accumulation in AS patients. (93pp)
AB Using **alpha-1,beta-1 integrin** receptor-inhibitor (I) and transforming growth factor-beta-1-inhibitor (II) for therapy of **kidney** disorders is claimed. (I) is given to a patient to limit a **kidney** disorder. Also claimed are: delaying onset of and/or slowing progression of Alport Syndrome (AS) in a patient by **administering** an agent that inhibits signal transduction via an **alpha-1, beta-1 integrin** receptor (A1B1IR) of a **kidney** cell or by blocking an A1B1IR binding site on the surface of a patient's **kidney** cell; delaying onset of and/or slowing progression of **kidney** disease in insulin-dependent diabetes mellitus (IDDM) in a patient by **administering** an agent that inhibits signal transduction via an A1B1IR of a **kidney** cell; delaying onset of and/or slowing progression of a **kidney** disease in IDDM in a patient by blocking an A1B1IR binding site on the surface of a patient's **kidney** cell; limiting **kidney** fibrosis in a patient; a mouse model for **kidney** disease, where the mouse does not express normal **collagen** type 4 in glomerular basement membrane and does not express A1B1IR; drug screening; and limiting matrix accumulation in AS patients.. . .

10/099,573
6,92,325

L6 ANSWER 12 OF 39 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-07191 BIOTECHDS
TITLE: New anti-VLA-1 antibodies are useful for preventing or treating VLA-1-mediated immunological or inflammatory disorders, e.g. psoriasis, eczema, burns, dermatitis, and abnormal proliferation of hair follicle cells or fibrosis; vector-mediated recombinant protein gene transfer and expression in hybridoma cell culture for use in disease prevention and therapy
AUTHOR: LYNE P D; GARBER E A; SALDANHA J W; KARPUSAS M
PATENT ASSIGNEE: BIOGEN INC
PATENT INFO: WO 2002083854 24 Oct 2002
APPLICATION INFO: WO 2002-US11521 12 Apr 2002
PRIORITY INFO: US 2001-303689 6 Jul 2001; US 2001-283794 13 Apr 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-093009 [08]
AB DERWENT ABSTRACT:

NOVELTY - Anti-**VLA-1** antibody (Ab) where the light chain complementarity determining regions (CDRs) are defined by amino acid residues 24-33, 49-55, and 88-96 of a fully defined sequence of 106 amino acids (S1) given in the specification, and where the heavy chain CDRs are defined by amino acid residues 31-35, 50-65, and 98-107 of a fully defined sequence of 118 amino acids (S2) given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a composition comprising (Ab) and a carrier; (2) an isolated nucleic acid comprising a coding sequence for: (a) the sequence of S1 or S2; (b) the light chain or heavy chain of an antibody produced by hybridoma mAQC2 (ATCC accession number PTA3273); (c) light chain or heavy chain of an antibody produced by cell line hAQC2 (ATCC accession number PTA3275); (d) the heavy chain of an antibody produced by cell line haAQC2 (ATCC accession number PTA3274) or cell line hsAQC2 (ATCC accession number PTA3356); (e) residues 1-106 of a fully defined sequence of 213 amino acids (S3) given in the specification; or (f) residues 1-118 of a fully defined sequence of 447 amino acids (S4) given in the specification; (3) treating a subject with an immunological disorder mediated by **VLA-1** comprising **administering** to the subject the composition of (1); (4) determining the level of **VLA-1** in a tissue comprising contacting the tissue with (Ab), and detecting the binding of the antibody to the tissue; (5) cells of: (a) hybridoma mAQC2 (ATCC accession number PTA3273); or (b) cell line hAQC2 (ATCC accession number PTA3275), haAQC2 (ATCC accession number PTA3274), or cell line hsAQC2 (ATCC accession number PTA3356); (6) identifying an inhibitor of an I domain of an **integrin**; (7) an inhibitor of I domain of **integrin** identified; and (8) computers for producing a three-dimensional (3-D) representation.

BIOTECHNOLOGY - Preferred Antibody: The antibody comprises: (a) a light chain variable domain sequence of S1 and a heavy chain variable domain sequence of S2; (b) the same heavy and light chain polypeptide sequences as an antibody produced by hybridoma mAQC2 (ATCC accession number PTA3273), cell line hAQC2 (ATCC accession number PTA3275), cell line hsAQC2 (ATCC accession number PTA3356), or cell line haAQC2 (ATCC accession number PTA3274); (c) at least one of the following residues in its light chain: Q1, L4, P46, W47, or Y71, or at least one of the following residues in its heavy chain: D1, V12, S28, F29, A49, T93,or R94 (Kabat numbering convention); (d) a light chain variable domain sequence defined by amino acid residues 1-106 of S3 and a heavy chain variable domain sequence defined by amino acid residues 1-118 of S4; (e) mutations L234A and L235A (EU numbering system) in its heavy chain as compared with an unmodified antibody; or (f) mutation N297Q in its heavy chain (EU numbering system). The antibody is preferably a humanized antibody. The heavy chain is mutated at one or more of amino acid residues selected from the group of residues 234, 235, 236, 237, 297, 318, 320 or 322 (EU numbering system), which causes an alteration in an effector function while retaining binding to **VLA-1** as compared with an unmodified antibody. The antibody is mutated at an amino acid residue that is a glycosylation site to eliminate the glycosylation site.

Preferred Method: Identifying an inhibitor of an I domain of an **integrin** comprises: (a) using the structure coordinates of: (i) hAQC2 amino acids comprising at least 7 of light chain residues Asn30, Tyr48, Trp90, Ser91, Asn93 and Trp95, and heavy chain residues Ser30, Arg31, Trp47, Ser52, Gly53, His56, Tyr58, Phe99, Gly100 and Asp101 defined in the specification, or +/- a root mean square deviation from the backbone atoms of the hAQC2 amino acids of not more than 1.10 Angstrom; (ii) I domain amino acid residues Asp154, Ser156, Asn157, Ser158, Tyr160, Glu192, Gln218, Arg219, Gly220, Gly221, Arg222, Gln223, Thr224, Asp257, His261, Asn263, Arg291, and Leu294, defined in the specification; or (iii) at least three of I domain amino acids comprising residues Glu192, Gln218, Arg219, Gly220, and Gly221, defined in the specification, or +/- a root mean square deviation from the backbone atoms of the I domain amino acids not more than 0.30 Angstrom; to generate a 3-D structure of a binding site; (b) employing the 3-D structure to design or select a potential antagonist; (c) synthesizing the potential antagonist; and (d) contacting the potential antagonist with hAQC2 or I domain to determine the ability of the potential antagonist to interact with hAQC2 or I domain, where the ability of the potential antagonist to interact with hAQC2 indicates that the potential antagonist is an inhibitor of the I domain. Preferred Computer: The computer for producing a 3-D representation of: (a) a molecular complex comprising the set of structure coordinates of a complex of a chimeric I domain of an alpha1beta1 **integrin** RDELTAH and a humanized antibody hAQC2, defined in the specification, or a homologue of the molecular complex having a root mean square deviation from the backbone atoms of the amino acids of not more than 0.65 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises at least a portion of the structure coordinates of the complex, defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D representation; (b) a molecule or molecular complex comprising a first binding site defined by structure coordinates of hAQC2 amino acids comprising at least 7 of light chain residues Asn30, Tyr48, Trp90, Ser91, Asn93 and Trp95, and heavy chain residues Ser30, Arg31, Trp47, Ser52, Gly53, His56, Tyr58, Phe99, Gly100 and Asp101 defined in the specification, or a homologue of the molecule or molecular complex, which comprises a second binding site having a root mean square deviation from the backbone atoms of the hAQC2 amino acids of not more than 1.10 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises the structure coordinates of hAQC2 amino acids comprising at least 7 of light chain residues Asn30, Tyr48, Trp90, Ser91, Asn93 and Trp95, and heavy chain residues Ser30, Arg31, Trp47, Ser52, Gly53, His56, Tyr58, Phe99, Gly100 and Asp101 defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D representation; (c) a first binding site defined by structure coordinates of hAQC2 amino acids comprising at least 7 of light chain residues Asn30, Tyr48, Trp90, Ser91, Asn93 and Trp95, and heavy chain residues Ser30, Arg31, Trp47, Ser52, Gly53, His56, Tyr58, Phe99, Gly100 and Asp101 defined in the specification, or a second binding site having a root mean square deviation from the backbone atoms of the hAQC2 amino acids of not more than 1.10 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises the structure coordinates of hAQC2 amino acids comprising at least 7 of light chain residues Asn30, Tyr48, Trp90, Ser91, Asn93 and Trp95, and heavy chain residues Ser30, Arg31, Trp47, Ser52, Gly53, His56, Tyr58, Phe99, Gly100 and Asp101 defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable

data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D; (d) a molecule or molecular complex comprising a first binding site defined by structure coordinates of I domain amino acid residues Asp154, Ser156, Asn157, Ser158, Tyr160, Glu192, Gln218, Arg219, Gly220, Gly221, Arg222, Gln223, Thr224, Asp257, His261, Asn263, Arg291, and Leu294, defined in the specification, or a homologue of the molecule or molecular complex, which comprises a second binding site having a root mean square deviation from the backbone atoms of the I domain amino acids not more than 0.92 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises the structure coordinates of I domain amino acid residues Asp154, Ser156, Asn157, Ser158, Tyr160, Glu192, Gln218, Arg219, Gly220, Gly221, Arg222, Gln223, Thr224, Asp257, His261, Asn263, Arg291, and Leu294, defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D; (e) a first binding site defined by structure coordinates of I domain amino acid residues Asp154, Ser156, Asn157, Ser158, Tyr160, Glu192, Gln218, Arg219, Gly220, Gly221, Arg222, Gln223, Thr224, Asp257, His261, Asn263, Arg291, and Leu294, defined in the specification, or a second binding site of a homologue having a root mean square deviation from the backbone atoms of the I domain amino acids not more than 0.92 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises the structure coordinates of I domain amino acid residues Asp154, Ser156, Asn157, Ser158, Tyr160, Glu192, Gln218, Arg219, Gly220, Gly221, Arg222, Gln223, Thr224, Asp257, His261, Asn263, Arg291, and Leu294, defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D; (f) a molecule or molecular complex comprising a first binding site defined by structure coordinates of I domain amino acids comprising at least three of residues Glu192, Gln218, Arg219, Gly220, and Gly221, defined in the specification, or a homologue of the molecule or molecular complex, which comprises a second binding site having a root mean square deviation from the backbone atoms of the I domain amino acids not more than 0.30 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises the structure coordinates of I domain amino acids comprising at least three of residues Glu192, Gln218, Arg219, Gly220, and Gly221, defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D; or (g) a first binding site defined by structure coordinates of I domain amino acids comprising at least three of residues Glu192, Gln218, Arg219, Gly220, and Gly221, defined in the specification, or a second binding site having a root mean square deviation from the backbone atoms of the I domain amino acids not more than 0.30 Angstrom, comprises: (i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, which comprises the structure coordinates of I domain amino acids comprising at least three of residues Glu192, Gln218, Arg219, Gly220, and Gly221, defined in the specification; (ii) a working memory for storing instructions for processing the machine-readable data; (iii) a central processing unit coupled to (ii) and (i) for processing the machine-readable data storage medium for processing the machine readable data into the 3-D representations; and (iv) a display coupled to (iii) for displaying the 3-D.

ACTIVITY - Antiallergic; Antiinflammatory; Immunosuppressive; Antianemic; Antiarteriosclerotic; Antiasthmatic; Antithyroid; Vasotropic; Vulnerary; Cytostatic; Antipsoriatic; Dermatological; Antidiabetic; Antibacterial; Nephrotropic; Ophthalmological; Antimigraine; Antipyretic;

Antirheumatic; Antiarthritic; Neuroprotective; Osteopathic. Arthritis was induced in mice by injection of a cocktail of anti-collagen type II monoclonal antibodies (mAbs) on day 0, followed by LPS administration on day 3. Mice were then treated with either anti-alpha1 mAb or a soluble TNF receptor Ig fusion protein starting on day 4. Progression of arthritis was completely blocked in mice receiving anti-alpha1 mAb starting at day 4, when compared to mice receiving control hamster mAb starting at day 4. The degree of inhibition seen with therapeutic administration of anti-alpha1 mAb was complete and was equal to that seen with preventive treatment of anti-alpha1 mAb (started at day 0). In comparison, treatment with TNF receptor Ig fusion protein from day 4 onwards resulted in only a 60-70% inhibition in arthritic score when compared to control Ig fusion protein. Combined treatment of anti-alpha1 mAb and TNF receptor Ig fusion together was effective at completely inhibiting arthritic score.

MECHANISM OF ACTION - Integrin Antagonist Alpha 1 Beta 1.

USE - The anti-VLA-1 antibodies are useful for preventing or treating VLA-1-mediated immunological or inflammatory disorders, such as skin related conditions (e.g. psoriasis, eczema, burns, dermatitis, and abnormal proliferation of hair follicle cells), fibrosis (e.g. kidney or lung fibrosis), allergic rhinitis, respiratory distress syndrome, asthma, bronchitis, tendonitis, bursitis, fever, migraine headaches, gastrointestinal conditions (e.g. inflammatory bowel disease, Crohn's disease, gastritis, irritable bowel syndrome, colitis and colorectal cancer), vascular diseases (e.g. atherosclerosis), periarteritis nodosa, thyroiditis, aplastic anemia, Hodgkin's disease, rheumatic fever, osteoarthritis, autoimmune diseases (e.g. type I diabetes, myasthenia gravis, rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis), sarcoidosis, nephrotic syndrome, renal failure, Behcet's syndrome, polymyositis, gingivitis, hypersensitivity (e.g. delayed type hypersensitivity or immediate hypersensitivity), graft and transplant rejections, graft versus host disease, conjunctivitis, swelling occurring after injury, myocardial ischemia, or endotoxin shock syndrome. The structural information can be used for computational drug design.

ADMINISTRATION - Dosage is about 0.001-100 (preferably 0.1-50) mg/kg/day. Administration may be oral, topical, intravenous, subcutaneous, intraperitoneal, intramuscular, intrmedullary, intraarterial, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intraspinal, intracranial, locally at sites of inflammation or tumor growth, by inhalation, or by implantation of an infusion pump or a biocompatible sustained release implant into the subject.

EXAMPLE - Robertsonian mice were immunized intraperitoneally (i.p.) with 25 mug of purified human alpha1beta1 emulsified with complete Freund's adjuvant. The mouse with the highest anti-alpha1-I domain titer was boosted i.p. with 100 mug of alpha1beta1 three days prior to fusion, and intravenously with 50 mug of alpha1beta1 one day prior to fusion. Spleen cells were fused with FL653 myeloma cells at a 1:6 ratio and were plated at 100000 and 33000 per well into 96-well tissue culture plates. Supernatants were assessed for binding to the alpha1beta1 integrin by single color FACS. Supernatants from the resulting hybridomas were screened for binding to the alpha1 -I domain. Selected supernatants were tested for their ability to inhibit K562-alpha1 dependent adhesion to Collagen IV. K562-alpha1 cells were labeled with 2mM 2'7' (bis-2-carboxyethyl-5 and -6) carboxyfluorescein penta acetoxymethylester (BCECF; Molecular Probes) in DMEM containing 0.25% BSA at 37degreesC for 30 minutes. Labeled cells were washed with binding buffer and re-suspended in binding buffer plus 5 mM MgCl2 at a final concentration of 1 x 106 cells/ml. 50 mul of supernatant was incubated with an equal volume of 2 x 105 K562-alpha1 cells in wells of a 96-well plate. The plate was then centrifuged and the supernatants removed. Cells were re-suspended in binding buffer and transferred to wells of a collagen-coated plate and incubated for 1 hour at 37degreesC. Following incubation, the non-adherent cells were removed by washing three times with binding buffer. Nineteen hybridomas were initially identified, the supernatants

of which bound to human leukemia K562 cells expressing the alpha1beta1 **integrin** (K562-**alpha1**) and to the **alpha1**-I domain. The immunoglobulins were purified from each of these hybridomas and tested for the ability to block either K562-**alpha1** or **alpha1**-I domain binding to **collagen** IV. The mAbs produced by clones AEF3, BGC5, AQC2 and AJH10 bind the **alpha1**-I domain, only mAbs AJH10 and AQC2 inhibit **alpha1**-I domain-dependent, or K562-**alpha1** adhesi on to **collagen** IV. (248 pages)

AB DERWENT ABSTRACT:

NOVELTY - Anti-**VLA-1** antibody (Ab) where the light chain complementarity determining regions (CDRs) are defined by amino acid residues 24-33, 49-55, and 88-96. . . sequence of 447 amino acids (S4) given in the specification; (3) treating a subject with an immunological disorder mediated by **VLA-1** comprising **administering** to the subject the composition of (1); (4) determining the level of **VLA-1** in a tissue comprising contacting the tissue with (Ab), and detecting the binding of the antibody to the tissue; (5). . . number PTA3274), or cell line hsAQC2 (ATCC accession number PTA3356); (6) identifying an inhibitor of an I domain of an **integrin**; (7) an inhibitor of I domain of **integrin** identified; and (8) computers for producing a three-dimensional (3-D) representation.

BIOTECHNOLOGY - Preferred Antibody: The antibody comprises: (a) a light. . . 297, 318, 320 or 322 (EU numbering system), which causes an alteration in an effector function while retaining binding to **VLA** -1 as compared with an unmodified antibody. The antibody is mutated at an amino acid residue that is a glycosylation site to eliminate the glycosylation site. Preferred Method: Identifying an inhibitor of an I domain of an **integrin** comprises: (a) using the structure coordinates of: (i) hAQC2 amino acids comprising at least 7 of light chain residues Asn30,. . . a molecular complex comprising the set of structure coordinates of a complex of a chimeric I domain of an alpha1beta1 **integrin** RDELTAH and a humanized antibody hAQC2, defined in the specification, or a homologue of the molecular complex having a root. . . Vulnerary; Cytostatic; Antipsoriatic; Dermatological; Antidiabetic; Antibacterial; Nephrotropic; Ophthalmological; Antimigraine; Antipyretic; Antirheumatic; Antiarthritic; Neuroprotective; Osteopathic. Arthritis was induced in mice by **injection** of a cocktail of anti-**collagen** type II monoclonal antibodies (mAbs) on day 0, followed by LPS administration on day 3. Mice were then treated with either anti-**alpha1** mAb or a soluble TNF receptor Ig fusion protein starting on day 4. Progression of arthritis was completely blocked in mice receiving anti-**alpha1** mAb starting at day 4, when compared to mice receiving control hamster mAb starting at day 4. The degree of inhibition seen with therapeutic administration of anti-**alpha1** mAb was complete and was equal to that seen with preventive treatment of anti-**alpha1** mAb (started at day 0). In comparison, treatment with TNF receptor Ig fusion protein from day 4 onwards resulted in only a 60-70% inhibition in arthritic score when compared to control Ig fusion protein. Combined treatment of anti-**alpha1** mAb and TNF receptor Ig fusion together was effective at completely inhibiting arthritic score.

MECHANISM OF ACTION - **Integrin** Antagonist **Alpha 1 Beta 1**.

USE - The anti-**VLA-1** antibodies are useful for preventing or treating **VLA-1**-mediated immunological or inflammatory disorders, such as skin related conditions (e.g. psoriasis, eczema, burns, dermatitis, and abnormal proliferation of hair follicle cells), fibrosis (e.g. **kidney** or lung fibrosis), allergic rhinitis, respiratory distress syndrome, asthma, bronchitis, tendonitis, bursitis, fever, migraine headaches, gastrointestinal conditions (e.g. inflammatory bowel. . . be oral, topical, intravenous, subcutaneous, intraperitoneal, intramuscular, intrmedullary, intraarterial, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intraspinal, intracranial, locally at sites of **inflammation** or tumor growth, by inhalation, or by implantation of an infusion pump or a biocompatible sustained release implant into the. . . immunized intraperitoneally (i.p.) with 25 mug of purified

human alpha1beta1 emulsified with complete Freund's adjuvant. The mouse with the highest anti-**alpha1**-I domain titer was boosted i.p. with 100 mug of alpha1beta1 three days prior to fusion, and intravenously with 50 mug. . . plated at 100000 and 33000 per well into 96-well tissue culture plates. Supernatants were assessed for binding to the alpha1beta1 **integrin** by single color FACS. Supernatants from the resulting hybridomas were screened for binding to the **alpha1** -I domain. Selected supernatants were tested for their ability to inhibit K562-**alpha1** dependent adhesion to **Collagen** IV. K562-**alpha1** cells were labeled with 2mM 2'7' (bis-2-carboxyethyl-5 and -6) carboxyfluorescein penta acetoxymethylester (BCECF; Molecular Probes) in DMEM containing 0.25% BSA. . . concentration of 1 x 106 cells/ml. 50 mul of supernatant was incubated with an equal volume of 2 x 105 K562-**alpha1** cells in wells of a 96-well plate. The plate was then centrifuged and the supernatants removed. Cells were re-suspended in binding buffer and transferred to wells of a **collagen**-coated plate and incubated for 1 hour at 37degreesC. Following incubation, the non-adherent cells were removed by washing three times with. . . binding buffer. Nineteen hybridomas were initially identified, the supernatants of which bound to human leukemia K562 cells expressing the alpha1beta1 **integrin** (K562-**alpha1**) and to the **alpha1**-I domain. The immunoglobulins were purified from each of these hybridomas and tested for the ability to block either K562-**alpha1** or **alpha1**-I domain binding to **collagen** IV. The mAbs produced by clones AEF3, BGC5, AQC2 and AJH10 bind the **alpha1**-I domain, only mAbs AJH10 and AQC2 inhibit **alpha1** -I domain-dependent, or K562-**alpha1** adhesi on to **collagen** IV. (248 pages)

L6 ANSWER 4 OF 39 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1998:28322861 BIOTECHNO
TITLE: Glomerular extracellular matrix components and integrins
AUTHOR: Sterk L.M.Th.; De Melker A.A.; Kramer D.; Kuikman I.; Chand A.; Claessen N.; Weening J.J.; Sonnenberg A.
CORPORATE SOURCE: L.M.Th Sterk, Division of Cell Biology, The Netherlands Cancer Institute, Amsterdam, Netherlands.
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AB It has become apparent that extracellular matrix components and their cellular receptors, the **integrins**, are important regulators of glomerular development and function. In this rapidly evolving field we studied the production of extracellular matrix components and **integrins** by rat glomerular visceral epithelial and mesangial cells, using molecular probes and antibodies that have recently become available. Special attention was paid to laminin isoforms and to splice variants of the **integrin** subunits α3 and α6.
Results were compared to the in **vivo** expression in human fetal, newborn and adult **kidneys**. The mesangial cells were found to produce laminin-1, nidogen and two as yet unidentified laminin isoforms with putative α chains of about 395 (αx) and of 375 kDa (αy), tentatively described before as bovine **kidney** laminin. Furthermore, they expressed the **integrins** . **alpha**.1.**beta**.1, .**alpha**.2. **beta**.1, .**alpha**.3A.**beta**.1, .**alpha**.5.**beta**.1, .**alpha**.vβ3, αvβ5, and small amounts of α6Aβ1 and α6Bβ1. The glomerular visceral epithelial cells produced the two new laminin isoforms mentioned above, laminin-5, but no laminin-1 or nidogen. The **integrins** α2. **beta**.1, . **alpha**.3A.**beta**.1, .**alpha**.6Aβ4, α6Bβ4 and the **integrin** subunit αv were found to be expressed. We show that during nephrogenesis, the laminin . **alpha**.1 chain disappears and is replaced by another α chain, possibly one of the two as yet unidentified α chains mentioned above. The laminin β1 chain is replaced by the β2 chain somewhat later in glomerular development. In general, the **integrins** found to be expressed in glomeruli of adult **kidney** were consistent with those found in cultured glomerular visceral epithelial and mesangial cells. No splice variant switch of the **integrin** α3 or α6 subunits could be demonstrated during nephrogenesis. Our results suggest an important role for the mesangial cell in providing nidogen as a crucial component of the supramolecular stucture of the glomerular basement membrane. Furthermore our results indicate that laminin αxβ2γ1 and αyβ2γ1 isoforms are important in the glomerulus of adult **kidney** and that the **integrin** α3Aβ1 is the main **integrin** receptor for laminin isoforms on glomerular visceral epithelial and mesangial cells, both in vitro and in **vivo**.
AB It has become apparent that extracellular matrix components and their cellular receptors, the **integrins**, are important regulators of glomerular development and function. In this rapidly evolving field we studied the production of extracellular matrix components and **integrins** by rat glomerular visceral epithelial and mesangial cells, using molecular probes and antibodies that have recently become available. Special attention was paid to laminin isoforms and to splice variants of the **integrin** subunits α3 and α6.
Results were compared to the in **vivo** expression in human fetal, newborn and adult **kidneys**. The mesangial cells were found to produce laminin-1, nidogen and two as yet unidentified laminin isoforms with putative α chains of about 395 (αx) and of 375 kDa

(αy), tentatively described before as bovine **kidney** laminin. Furthermore, they expressed the **integrins** . **alpha.1.beta.1**, .**alpha**.2. **beta.1**, .**alpha**.3A.**beta.1**, .**alpha**.5.**beta.1**, .**alpha**.vβ3, αvβ5, and small amounts of α6Aβ1 and α6Bβ1. The glomerular visceral epithelial cells produced the two new laminin isoforms mentioned above, laminin-5, but no laminin-1 or nidogen. The **integrins** α2. **beta.1**, . **alpha**.3A.**beta.1**, .**alpha**.6Aβ4, α6Bβ4 and the **integrin** subunit αv were found to be expressed. We show that during nephrogenesis, the laminin . **alpha**.1 chain disappears and is replaced by another α chain, possibly one of the two as yet unidentified α chains mentioned above. The laminin β1 chain is replaced by the β2 chain somewhat later in glomerular development. In general, the **integrins** found to be expressed in glomeruli of adult **kidney** were consistent with those found in cultured glomerular visceral epithelial and mesangial cells. No splice variant switch of the **integrin** α3 or α6 subunits could be demonstrated during nephrogenesis. Our results suggest an important role for the mesangial cell in. . . glomerular basement membrane. Furthermore our results indicate that laminin αxβ2γ1 and αyβ2γ1 isoforms are important in the glomerulus of adult **kidney** and that the **integrin** α3Aβ1 is the main **integrin** receptor for laminin isoforms on glomerular visceral epithelial and mesangial cells, both in vitro and in **vivo**.